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(54) Title: METHODS FOR THE PRODUCTION AND USE OF ANTI-IDIOTYPIC ANTIBODIES USING HUMAN Ig EXPRESSING TRANSGENIC ANIMALS

(57) Abstract

Methods for production of enriched populations of anti-idiotypic antibodies are provided. These antibodies are produced by immunizing transgenic non-human animals. The transgene utilized in these animals encodes and, in the animals, expresses an immunoglobulin protein which is idiotypically different from the immunizing immunoglobulin. The animals therefore produce virtually no antibodies against constant region determinants on the immunoglobulin expressed by the transgene. A preferred method will produce enriched populations of monoclonal anti-idiotypic antibodies utilizing hybridomas formed by fusion of splenocytes taken from the transgenic animals of the invention.

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METHODS FOR THE PRODUCTION AND USE OF ANTI-IDIOTYPIC ANTIBODIES USING HUMAN Ig EXPRESSING TRANSGENIC ANIMALS

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BACKGROUND OF THE INVENTION

1. *Field of the Invention*

This invention relates to the use of transgenic animals to produce human anti-idiotypic antibodies. Because the animals are tolerant to the constant regions of human Ig they can be used to produce enriched populations of anti-idiotypic antibodies (in particular, monoclonal antibodies "mAb's") and to test the potential toxicity of humanized or chimeric antibodies.

2. *Description of Related Art*

An anti-idiotypic antibody is an antibody which recognizes unique determinants present on another antibody of a different idiotype. These determinants are located in the variable region of the antibody. It is this region which binds to a given epitope and, thus, it is responsible for the specificity of the antibody. Anti-idiotypic antibodies can be used in a variety of diagnostic, therapeutic and research applications. For example, anti-idiotypic antibodies can be important to the diagnosis and treatment of certain autoimmune diseases (where an antibody reacts with an antigen or "self-antigen" which is a normal constituent of the host). To illustrate, this reaction occurs when leukemia cells from chronic lymphocytic leukemia (CLL) patients express monoclonal immunoglobulin (Ig) class M autoantibodies which are specifically reactive for self-IgG. Ig autoantibodies are also expressed by cells active in other lymphoproliferative diseases, such as lymphoma and Sjorgen's syndrome. These autoantibodies

are associated with cross-reactive idiotypes (CRI's); i.e., they have idiotypic determinants which occur on antibodies other than the antibody which stimulated the production of that anti-idiotypic antibody.

If one could produce monoclonal antibodies which react with the different autoantibody idiotypes associated with a given autoimmune disease, one could readily target and identify the cells which express those antibodies for therapeutic and diagnostic purposes. However, effective therapy and diagnosis of autoimmune disease not only requires production of antibodies (preferably mAb's) specific for idiotypic determinants on autoantibodies produced at a given time by a given cell population, it also requires that a supply of antibodies reactive to other idiotypes be available. One reason for this need is the ability of diseased cells to avoid immune attack by losing idiotypic determinants and acquiring others (see, e.g., Carson, *et al., eds., "Anti-Idiotypic Therapy of Leukemias and Lymphomas"*, Idiotypes in Biology and Medicine; Chem. Immunol. Basel, Karger, Vol. 48, pp 126-166, 1990).

Currently, the process used for production of anti-idiotypic monoclonal antibodies is both laborious and time-intensive. Generally, anti-human, anti-idiotypic antibodies are produced by immunization of animals (commonly mice) with human 1g paraproteins (i.e., a homogenous or monoclonal Ig derived from an expanded clone of plasma cells, also referred to as a myeloma protein). The immune system of the immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants. However, normal animals will usually also produce an immune response which is directed against constant region determinants on the Ig. As a result, following fusion of splenocytes from these mice with myeloma cells, selection of mAb-producing hybridomas

specific for idiotype is complicated by the presence of clones reacting with constant region determinants.

To avoid this problem, the fusion is either distributed between many tissue culture wells (to limit the number of clones produced in each well), or is distributed to fewer wells but followed by rapid cloning of positive clones in each well. Either way, the process is labor-intensive.

Clearly, an improved process which allows for relatively rapid, large-scale production of antibodies specific for a variety of idiotypic determinants would be of significant value. A need, therefore, exists for means to readily develop enriched populations of anti-idiotypic antibodies, i.e., populations which contain few (if any) anti-constant region antibodies. The present invention addresses that need through the development and use of transgenic non-human animals (preferably, mice) which are tolerant to the constant regions of human Ig proteins.

SUMMARY OF THE INVENTION

The invention consists in one aspect of transgenic non-human animals which express human Ig proteins. When subsequently immunized with idiotypically different Ig, the mice are tolerant to the Ig constant regions and determinants shared by the transgene as immunizing Ig, but produce antibodies against any idiotypic determinants present on the immunizing antibody. As used in this context, "idiotype" refers to determinants on the immunizing antibodies which are structurally different in their variable region than the Ig expressed by the transgene and at least substantially similar in their constant regions. "Substantially similar" in this context refers to constant regions of different Ig which are identical or, if not identical, are not detectably cross-reactive.

According to one embodiment of the invention, splenocytes taken from the transgenic animals are used to produce mAb's which are specific for idiotypic determinants on human antibodies. These mAb's can be used to identify idiotypic determinants on surface Ig expressed by lymphoproliferative cells and can specifically target those determinants in immunotherapy.

In another aspect of the invention, the transgenic animals serve as an animal model for determining the potential toxicity of humanized or chimeric antibodies. Because the transgenic animals are tolerant to constant region determinants of human Ig, their immune response can be expected to reflect the immunogenicity of the variable regions of the antibodies, in particular the human or chimeric regions. More specifically, antibodies to the humanized antibodies are detected and their production levels compared to a background determined by immunization with an Ig which is the same except for the absence of the humanized or chimeric region. Preferably, the immunizing Ig's will be mAb's.

In another aspect of the invention, the transgenic animals are used to produce anti-idiotypic reagents useful in identifying idiotypes associated with diseases, such as those which are characterized by high titers of rheumatoid factors (anti-IgG autoantibodies, hereafter "RF") in serum. This aspect of the invention can be of particular use in dissecting the RF repertoire present in patients with rheumatoid arthritis, where RF's are not significantly restricted in their usage of light or heavy chain variable regions.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts the results of an enzyme-linked immunosorbent assay (ELISA) demonstrating inhibition of serum binding to GLO protein coated microtiter plate wells. Black columns in the figure represent the percentage inhibition of binding of sera from transgenic

mice immunized with GLO. White columns represent the percentage inhibition of binding of sera from a non-transgenic mouse immunized with polyclonal human IgM. These sera were used for inhibition studies at a concentration which has been determined to represent the linear portion of the binding curve to GLO protein. The inhibitory antigens (2 ug/ml each) are identified along the X axis of the figure

FIGURE 2 depicts the results of an ELISA to detect binding of sera from GLO immunized mice to other IgM paraproteins. AB29 transgenic mice immunized with GLO are denoted by open circles in the figure. Control non-transgenic littermates immunized with GLO are denoted by closed circles. Means of triplicate results are shown by horizontal bars. Results are expressed as arbitrary units/ml. 1000 units/ml is the level of binding to human IgM shown by serum from a control mouse immunized with human IgM (data not shown).

DETAILED DESCRIPTION OF THE INVENTION

A. Suitable Animal Recipients of the Transgenes of the Invention.

The methods and antibodies of the invention derive from the use of an animal which expresses a transgene encoding human Ig antibodies. This animal will be any nonhuman animal which can produce a humoral or cell-mediated immune response, including mammals, reptiles and amphibians. Preferably, the animal which receives the transgene will be a rodent. Most preferably, this rodent will be a mouse. For simplicity, therefore, the animal recipient of the transgene will be described hereafter as a mouse (but the invention will be understood to not be limited to the use of mice).

B. Introduction of the Transgenes of the Invention into an Animal Recipient

Introduction of an Ig encoding transgene chosen as taught herein into a mouse or mice will be

accomplished using techniques which are well-known in the art. These techniques are summarized below with, where appropriate, citations to references which provide additional details concerning application of the techniques. Those of ordinary skill in the art will be familiar with these applications and will be able to apply the techniques in the context of the present invention without undue experimentation.

For example, embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for microinjection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2/pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster, *et al.*, *Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic mouse will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating transgenes in practicing the invention.

Retroviral infection can also be used to introduce a transgene into a mouse. The developing mouse embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Jaenich, *Proc. Natl. Acad. Sci USA* 73:1260-1264, 1976). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.*, *Manipulating the Mouse Embryo*,

Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner, *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:6927-6931, 1985; Vander Putten, *et al.*, *Proc. Natl. Acad. Sci USA* 82:6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Vander Putten, *supra;* Steward, *et al.*, *EMBO J.*, 6:383-388, 1987).

Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner, *et al.*, *Nature*, 298:623-628, 1982). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic non-human animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner, *et al.*, *supra*, 1982).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans, *et al.*, *Nature*, 292:154-156, 1981; Bradley, *et al.*, *Nature*, 309:255-258, 1984; Gossler, *et al.*, *Proc. Natl. Acad. Sci USA*, 83:9065-9069, 1986; and Robertson, *et al.*, *Nature*, 322:445-448, 1986). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction. These transformed ES cells can thereafter be combined with blastocysts from a mouse. The ES cells will thereafter colonize the embryo

and contribute to the germ line of the resulting chimeric animal (see for review, Jaenishch, *Science*, 240:1468-1474, 1988). Where a transgenic animal is maintained by cross-mating with another strain, positive progeny of those matings can be confirmed by an assay for the protein expressed by the transgene in sera or another suitable assay sample. Immunoassays using anti-transgene product assays are particularly preferred for this use and can be constructed without undue experimentation by those of ordinary skill in the art.

C. Suitable Transgenes for Use in the Invention

Generally, the transgene will be a mammalian DNA or cDNA nucleic acid having a nucleotide sequence therein which encodes an Ig protein (preferably an IgM or IgG protein). The transgene is introduced into the germline of mice by utilizing the methods described above. From a functional perspective, when expressing the transgene are injected with Ig proteins of an idiotype different than the idiotype of the Ig encoded by the transgene, the transgenic mice will produce antibodies which are specific for variable region (but not shared region) determinants on the immunizing Ig. Preferably, the transgene will be a human DNA or cDNA nucleic acid.

The transgenes of the invention will also be those which do not, when expressed, result in a compromise of the recipient's immune system sufficient to endanger its immune competence. While it would not be possible to precisely define the point at which the recipient's immune system is at risk, those skilled in the immunological art will recognize that the immune competence of the recipient of the transgene can be monitored through use of commonly known techniques. For example (as illustrated further in the Examples below), levels of endogenous Ig in serum and the antibody response to known antigens can be quantified using conventional

immunoassays compared to a "normal" background value to determine if the recipient's antibody response substantially varies from that value.

The potential for immune compromise resulting from use of a particular transgene can also be predicted (and avoided) to some extent by (1) determining whether the Ig product of the transgene reacts with the recipient's endogenous Ig and molecules, and (2) choosing a transgene whose Ig product is substantially unreactive with the recipient's Ig as self-components. Techniques for determining whether the transgene Ig product will cross-react with the recipient's Ig are well-known in the art; examples illustrating how this determination can be made are provided herein. Generally, suitable transgenes for use in the invention will not produce Ig that detectably or significantly react with endogenous Ig as self components.

A "detectable" reaction with endogenous Ig means a reaction which results in a compromise of the recipient's immune system sufficient to endanger its immune competence.

Although, as discussed further below, it is possible that tolerance to constant region determinants on Ig which are structurally different from the transgene-encoded Ig, can be induced using a transgene encoding Ig of any class, IgM and IgG encoding transgenes are preferred. This preference is based on their greater concentration in normal serum and for the role (discussed *supra*) these Ig play in autoimmune diseases.

Particularly useful IgM encoding transgenes for use in the invention will be selected from the family of genes known to encode rheumatoid factors (RF). RF's are typically IgM autoantibodies directed against IgG which are expressed on the surface of lymphocytes. In healthy human subjects, an increase in RF precursor B cells

coincides with the normal secondary immune response to antigen. However, high titers of serum RF are also characteristic of human autoimmune diseases such as mixed cryoglobulinemia, Sjorgen's Syndrome and rheumatoid arthritis (RA). Approximately 95% of CLL cells also express surface RF's.

The RF transgene chosen should not detectably cross-react with immunoglobulins of the mouse. Germline-encoded autoantibodies will characteristically be polyspecific and can be expected to cross-react with specific mouse immunoglobulins. The RF transgene chosen, therefore, will preferably express a monoclonal RF. One such RF is the Les IgM RF.

The Les IgM RF is an anti-human IgG autoantibody which shows a number of point mutations from germline and apparently does not cross-react with mouse IgG. This RF is found in the sera of human patients who suffer from autoimmune disease and share cross-reactive idiotypes (CRI). The Les kappa (K) gene is derived from a conserved variable region gene that often encodes light chains of human autoantibodies.

Genes encoding other monoclonal RF's may also be suitable candidates for use as transgenes in the methods of this invention. However, because the full-length genes encoding for the heavy and light chains of the Les RF are known, it can be readily used as the transgene in the methods of this invention. The nucleotide sequence for the Les K light chain gene has been published in, for example, in Jirik, *et al.*, *Proc. Natl. Sci. USA*, 83:2195-2199 (1986). The nucleotide sequence for the Les heavy chain gene has been published in, for example, Roudier, *et al.*, *J. Immunol.*, 144:1526-1530 (1990).

Unexpectedly, the transgenic mice created according to the method of the invention were not only tolerant to the constant region of human IgM which had variable

regions structurally different from the transgene, but were also produced endogenous serum Ig at substantially normal levels. Whether or not this tolerance occurs at the B or T cell level in these mice is not known, but it was a substantially consistent trait of the mice which received an IgM encoding transgene as described herein. Based on this response, it would be reasonable to predict that mice receiving an IgG, IgA, IgE or IgD encoding transgene would also be tolerant to the constant region of idiotypically different Ig proteins.

25 samples of fertilized ova from one strain of transgenic mice (AB29) produced according to the method of the invention have been deposited with the American Type Culture Collection, 1301 Parklawn Drive, Rockville, MD on December 22, 1993 (Accession No. 72014). No admission that this deposit was necessary to enablement of the claims herein is made or intended.

The deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable ova for 30 years from the date of deposit. The ova will be made available by ATCC under the terms of the Budapest Treaty which assures permanent and unrestricted availability of the progeny of the ova to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C., §122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if the ova deposited should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same ova. Availability of a deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The written specification herein is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope to the strain deposited, since the deposited embodiment is intended as a single illustration of one aspect of the invention and the use of any transgenic strain that is functionally equivalent is within the scope of this invention.

D. <u>Isolation or Synthesis of Suitable Transgenes.</u>

Generally, transgenes for use in the invention can be obtained by several methods which are well known to those skilled in the art. For example, the DNA of the transgene can be isolated using hybridization procedures which are well known in the art. These include, but are not limited to: 1) hybridization of probes to genomic or cDNA libraries to detect shared nucleotide sequences; 2) antibody screening of expression libraries to detect shared structural features and 3) synthesis by the polymerase chain reaction (PCR).

Hybridization procedures are useful for the screening of recombinant clones by using labeled mixed synthetic oligonucleotide probes where each probe is potentially the complete complement of a specific DNA sequence in the hybridization sample which includes a

heterogenous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to the single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).

A cDNA library containing the transgene(s) of interest can be screened by injecting the various cDNAs into oocytes, allowing sufficient time for expression of the cDNA gene products to occur, and testing for the presence of the desired cDNA expression product by, for example, using an antibody or antibodies specific for the rheumatoid factors described below or by using functional assays for rheumatoid factor activity and a tissue expression pattern characteristic of rheumatoid factors. Alternatively, a cDNA library can be screened indirectly for Ig encoding transgenes having at least one epitope using antibodies specific for the Ig. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of the Ig cDNA of interest.

Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This

requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogenous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).

The development of specific DNA sequences for use as the transgene can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codon for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated form a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA. Of these three methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid

residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

E. Immunization to Stimulate Production of Anti-Idiotypic Antibodies

The transgenic mice of the invention are preferably immunized with an idiotypic Ig to stimulate production of anti-idiotypic antibodies. Preferably, the constant region of the immunizing Ig antigen will be substantially similar to the constant region of the Ig encoded by the transgene. "Substantially similar" in this context means that the constant region determinants on the Ig will be immunologically cross-reactive. The immunizing Ig will, however, differ from the transgene-encoded Ig in its variable region and will react with (i.e., be recognized as an antigen by) the transgene-encoded Ig.

A multiple injection immunization protocol is preferred for use in this regard (see, e.g., Langone, *et*

al., eds., "Production of Antisera with Small Doses of Immunogen: Multiple Intradermal Injections", Methods of Enzymology (Acad. Press, 1981). For example, a good anti-idiotypic response can be obtained in mice by intradermal injection of 50 μg of the Ig idiotypic antigen emulsified in Complete Freund's Adjuvant followed three weeks later by a boost of the same antigen in incomplete Freund's adjuvant. Those of ordinary skill in the art will be able to readily modify the time course of immunization and dosages of antigen without undue experimentation; e.g., by administering additional booster immunizations, shortening or lengthening the time between immunizations, administering antigen without adjuvant, and equivalent techniques known in the art. Immunization may also be performed using any suitable parenteral route of administration, although intraperitoneal, intradermal or intravenous routes are preferred.

F. Production of Anti-Idiotypic Monoclonal Antibodies.

The invention consists in one aspect of monoclonal, anti-idiotypic antibodies against Ig (preferably IgM and/or IgG) proteins which differ structurally from the protein encoded by the transgene (hereafter, unless context otherwise requires, the mAb's of the invention").

The term "antibody" as used in this invention is meant also to include intact molecules as well as fragments thereof, such as for example, Fab and $F(ab')_2$, which are capable of binding the epitopic determinant.

The general method used for production of hybridomas secreting mAb's, is well known (Kohler and Milstein, *Nature*, 256:495, 1975). Briefly, as described by Kohler and Milstein the technique comprised lymphocytes isolated from regional draining lymph nodes of five separate cancer patients with either melanoma, teratocarcinoma or

cancer of the cervix, glioma or lung, were obtained from surgical specimens, pooled, and then fused with SHFP-1. Hybridomas were screened for production of antibody which bound to cancer cell lines.

According to the method of this invention, use of the transgenic mice as the source of splenocytes for production of hybridomas ensures that the hybridomas will secrete a population of anti-idiotypic antibodies which is substantially free of antibodies against constant region determinants (i.e., a population of antibodies enriched for anti-idiotypic antibodies).

Confirmation of anti-idiotypic specificity among antibodies in this enriched population (including specificity for a particular known idiotypic determinant) can be accomplished using relatively routine screening techniques to determine the elementary reaction pattern of the mAb of interest. Thus, if an mAb being tested binds with an idiotypic determinant on an Ig protein which differs structurally from the protein encoded by the transgene, then the antibody being tested and the antibody produced by the hybridomas of the invention are equivalent to one another.

It is also possible to evaluate an mAb to determine whether it has the same specificity as a mAb of the invention without undue experimentation by determining whether the mAb being tested prevents a mAb of the invention from binding to a known idiotypic determinant on an Ig protein of interest (hereafter, unless context otherwise requires, an "Ig idiotypic antigen"). If the mAb being tested competes with the mAb of the invention, as shown by a decrease in binding by the mAb of the invention, then it is likely that the two monoclonal antibodies bind to the same or a closely related idiotope.

Still another way to determine whether a mAb has the specificity of a mAb of the invention is to pre-incubate the mAb of the invention with the Ig idiotypic antigen with which it is normally reactive, and determine if the mAb being tested is inhibited in its ability to bind the antigen. If the mAb being tested is inhibited then, in all likelihood, it has the same, or a closely related, idiotypic specificity as the mAb of the invention.

Once the idiotypic specificity of a mAb has been determined, it can be used to screen for other mAb's which recognize the same idiotypic determinant. Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same idiotypic determinant.

Other techniques to characterize the specificity of mAb's are known in the art and are suitable for use in the invention, such as immunohistochemical characterization (which technique is used in the Examples below to characterize the anti-CRI specificity of certain mAb's).

G. <u>Purification of Polyclonal Anti-Idiotypic Antibodies</u>

If desired, polyclonal anti-idiotypic antibodies produced by the transgene recipient according to the methods of the invention may also be isolated. These antibodies may be purified according to means well-known to those skilled in the immunological arts, such as binding to and elution from a matrix to which the Ig recognized by the anti-idiotypic antibodies is bound.

H. <u>Detection of Anti-Idiotypic Antibody Production By the Immunized Transgene Recipient</u>

For certain purposes, it may be desirable to detect the presence and quantity of anti-idiotypic antibodies

produced by the immunized transgene recipient. For example, the immunogenicity of a humanized or chimeric antibody (which is idiotypically different from the Ig encoded by the transgene) can be tested by immunizing the transgenic mice of the invention. "Humanized" antibodies are known in the art to include murine variable region human constant region chimeras and human frameworks with murine complementary determining regions grafted thereon (see, e.g., Cabily, et al., Proc. Nat'l. Sci. Acad. USA, 81:3273, 1984), and Riechmann, *et al.*, *Nature*, 332:323, (1988).

In this application, anti-idiotypic antibodies which are produced against the humanized or chimeric regions of interest are detected in a biological sample from the immunized transgene recipient. Examples of types of immunoassays which can detect anti-idiotypic antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the anti-idiotypic antibodies Ig or fragments thereof of known idiotypic specificity can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other suitable immunoassay formats without undue experimentation.

As a control, transgenic mice of the invention may be immunized with an Ig which is of the same idiotype and isotype as the humanized Ig except for the absence of the humanized region. This control will provide a background value for comparison to evaluate the immunogenicity of the humanized region. This information can be used to evaluate the potential toxicity of a humanized antibody.

Detection and characterization of the idiotypes which the transgene recipient has produced antibodies against can also be useful in determining the repertoire of idiotypes which are associated with a particular disease. In this regard, anti-idiotypic antibodies whose binding specificity has been characterized as described herein may be used as reagents to characterize the specificity of Ig produced in patients, particularly those who are suffering from an autoimmune disease such as rheumatoid arthritis.

For example, a biological sample taken from a patient who has been diagnosed as having, or is suspected of having, a disease known to be associated with antibodies of a particular idiotype can be passed through or over a solid phase to which anti-idiotypic antibodies of this invention are bound for use as reagents. The biological sample will be one in which the antibodies of interest may be detectably present (such as serum). Binding of anti-idiotypic antibodies of known specificity can then be detected utilizing detection means well-known in the art such as those which are described elsewhere herein; *in vivo* detection techniques known to those skilled in the art may also be utilized with attachment of a detectable label to the anti-idiotypic antibody reagent.

This approach can also be used to evaluate the effectiveness of a particular course of immunotherapy by determining whether targeted cells may be "escaping" therapy by abandoning targeted idiotypic determinants in favor of others. The enriched populations of anti-idiotypic antibodies produced according to the methods of the invention will also create a ready supply of diagnostically effective antibodies for use *in vivo* or *in vitro*.

Examples illustrating the methods of the invention are provided below to illustrate their practice. These examples should not, however, be construed as limiting the invention.

EXAMPLE I

PREPARATION OF TRANSGENE CONSTRUCTS

The heavy and light chain genes that encode the human Les IgMRF (see, SEQ I.D. No. 1-4) were isolated from RF-expressing B cells which were positive for CLL.

To that end, peripheral blood lymphocytes (>85% malignant cells) were obtained, prior to antineoplastic drug therapy, from a 74-year-old woman with CLL associated with ulcerating cutaneous vasculitis of the lower extremities and with a monoclonal IgM cryoglobulin with RF activity. The CLL cells were either used immediately or cryopreserved in liquid nitrogen.

The CLL cells were conventionally fused with the hypoxanthine phosphoribosyltransferase (HPRT)-deficient WIL2-729-HF_2 lymphoblastoid B-cell line as described in Heitzmann, *et al.*, *Monoclonal Antibodies and Cancer;* Boss, *et al.*, eds. (Academic Press, 1983) pp. 157-162, except that the CLL cells were not stimulated *in vitro*. The WIL2-729-HF_2 cells were provided by R. Lundak, formerly at University of California, Riverside. In brief, 1.7×10^7 washed CLL cells were fused with 4.4×10^7 WIL2-729-HF_2 cells (fusion ration 1:2.5) and were plated at either 1.75×10^5 or 2.5×10^5 cells per well in microwell trays containing murine peritoneal macrophage feeder layers. Control wells contained nonfused CLL cells, WIL2-729-HF_2 cells, or mixtures of these cells. Hypoxanthine/aminopterin/thymidine (HAT) selective medium was added to all wells. Two to three weeks after fusion, supernatants were harvested from control and fusion wells and tested for IG and IgM RF secretion by enzyme-linked immunosorbent assay (ELISA). RF-positive wells were then expanded, subcloned by limiting dilution, and retested for RF activity.

The synthesis of multiple synthetic peptides corresponding to the individual CDR structures in the light and heavy chains of different IGM RF paraproteins and the preparation of antipeptide antibodies has been described in detail in the art (see, e.g., Chen, *et al.*, *J. Exp. Med.*, 159:1502-1511). Antipeptide antibodies were raised by immunizing rabbits with peptide-KLH conjugates emulsified in Freund's complete adjuvant.

Serum IgM RF was purified by repeated cryoprecipitation, followed by chromatography over "SEPHADEX G-200" in 0.1 M acetic acid. The purified IgM RF was analyzed with the multiple antipeptide antibodies by immunoblotting.

CLL cell DNA was then partially digested with *Mbo* I (New England Biolabs). Fragments of 10-20 kilobases (KB) long, obtained by sucrose density centrifugation, were ligated with bacteriophage T4 DNA ligase (New England Biolabs) to the *Bam*HI sites of the phage EMBL3 (described in Frischauf, *et al., J. Mol. Biol.* 170:827-842).

After initial cloning of the ligated DNA into the lambda Dash expression vector (Stratagene, La Jolla, CA) a light chain construct was prepared as follows. A 13kb Sal 1 fragment containing the VkIIIa (Vk328) gene and kappa constant region, 3.1 kb of 5' sequences and 5.0kb of 3' sequences was subcloned into the well-known plasmid pUC18.

A Les heavy chain construct was also prepared by initial cloning into the lambda Dash vector. Then, a 15kb Sal 1 fragment containing the VhIV gene and Cu constant region together with 0.6kb of 5' sequences and 1.5kb of 3' sequences was subcloned into pSVG-gpt (although other plasmid vectors would also be suitable, this vector was used as a gift from Dr. Christopher Goodnow, Stanford University, Stanford, CA). HindIII linkers were added onto a 0.8kb Eco RI restriction

fragment containing the murine immunoglobulin heavy chain enhancer; this fragment was cloned into a HindIII site on the heavy chain plasmid between the variable constant and regions just 5' of the switch sequence.

EXAMPLE II

INTRODUCTION OF TRANSGENE INTO MICE

Heavy and light chain genes coding for the human IgM RF were excised from the vector sequences described in Example I by Sal1 digestion. The inserts were purified on a 10-50% sucrose density gradient followed by extensive dialysis against 5mM Tris, 0.1mM EDTA pH7.5 buffer. For microinjection, the concentration of the fragment was adjusted to 2μg/ml total DNA containing equimolar concentrations of the heavy and light chain constructs. Ova used for microinjection were derived from matings (C57BL/6 x SJL/J) F_2 mice (Scripps Clinic and Research Foundation, La Jolla, CA). The founder lines were then maintained by backcross mating with C57B1/6 or MRL/lpr strains of mice (The Jackson Laboratory, Bar Harbor, ME).

Positive progeny of transgenic matings were identified by measuring the level of human IgM RF in the serum of 4-5 week old mice. Plates were coated with human IgG (Cappel Laboratories, Durham, NC) at 10 μg/ml, and binding of serial dilutions of mouse serum was determined by sequential addition of anti-human IgM biotin (Accurate Scientific, Co., Westbury, NY), streptavidin-peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and peroxidase substrate (KPL). Absorbance was measured at 450 nm. Levels of expression were determined by comparison with a standard curve of binding by purified Les IgM RF.

Levels of mouse Ig in serum and culture supernatants were determined by coating plates with affinity-purified goat anti-mouse Ig (KPL) and detected with affinity-

purified goat anti-mouse Ig-biotin (Jackson Immunoresearch, West Grove, PA).

Of the seven founder lines which were modified with the transgene and had human IgM RF in their serum which did not detectably cross-react with mouse Ig, two were found to simultaneously express RF (i.e. from the heavy and light chain genes) expression of both on the surface of B cells. These two strains were respectively named AB8 and AB29.

Specifically, approximately 15% of AB8 splenic B cells and 70% of AB29 splenic B cells express human RF on the cell surface. As compared to control (nontransgenic) littermates, expression of mouse Ig by all splenic B cells was reduced by 85%, presumably as a result of allelic exclusion.

EXAMPLE III

PHENOTYPE OF SPLEEN CELLS FROM AB29 TRANSGENIC MICE

To illustrate the phenotype of human RF-expressing B cells in these mice, the percentage of splenic B cells from the AB29 mice which express various Ig and markers on the cell surface are summarized in Table I below.

There are 7 mice in each group discussed in the Table unless otherwise indicated. The results shown are the mean percentages of total splenic mononuclear cells that express each antigen with ranges shown in parenthesis. The data was obtained by fluorescence analysis as described below.

Percentage of total cells

Cell surface marker	AB29 mice (n = 7)	Control littermates (n = 7)
B cells	55.4 (43-67)	69.6 (57-78)
Human IgM	39.1 (28-55)	0.0
Human κ	40.6 (26-53)	0.0
Mouse IgM	10.7 (5.24)	60.6 (46-78)
Mouse κ	10.3 (6-22)*	64.7 (52-74)
Mouse IgD	4.8 (3-10)*	52..2 (39-64)* n=6
T cells (CD4 + CD8)	35.0 (24-43)+	20.5 (20-29)+ n=5

Spleen cells for fluorescence analysis were separated on Lymphocyte M gradients (Accurate Scientific Co.). Cell phenotype was determined using the following reagents: goat anti-human IgM-FITC (Jackson Immunoresearch); anti-B220-PE (Pharmingen, San Diego, CA); anti-mouse IgD-PE (Pharmingen); anti-mouse k-PE (Fisher Scientific Co., Pittsburgh, PA); anti-mouse CD5-PE (Boehringer Mannheim Biochemicals, Indianapolis, IN); goat anti-human k-biotin (Sigma Chemical Co., St. Louis, MO); goat anti-mouse IgM-biotin (Jackson Immunoresearch); goat anti-mouse IgG-biotin (Kirkegaard & Perry Laboratories); rat anti-mouse CD4-biotin (YTA3.1); rat anti-mouse CD8-biotin; streptavidin-PE (Molecular Probes, Eugene, OR); and streptavidin-FITC (Molecular Probes). Cell phenotypes were analyzed on a FACSCAN flow cytometer (Becton Dickinson & CO., San Jose, CA), using techniques well-known to those skilled in the art.

EXAMPLE IV

IMMUNIZATION OF AB8 AND AB29 MICE WITH HUMAN IgM

Each group of 3 AB8 and 3 AB29 mice together with control non-transgenic littermates (described in Example II) were immunized intraperitoneally (i.p.) with 50 μg of human IgM in saline emulsified 1:1 with Complete Freunds Adjuvant to a total volume of 200 μl. Each mouse received an i.p. booster injection 3 weeks later of 25 μg human IgM and saline in Incomplete Freunds Adjuvant (1:1 emulsion to 200 μl volume). A third booster injection was given more than 2 months later 50 μg human IgM in saline administered intravenously without adjuvant.

EXAMPLE V

GENERATION OF RF SECRETING HYBRIDOMAS

Spleens were removed from AB29 transgenic mice and then teased apart in serum-free IMDM (Irvine Scientific, Santa Ana, CA). 10^8 spleen cells were combined with 6 x 10^7 NSO myeloma cells (a common fusion partner which does not produce endogenous Ig), washed in serum-free medium, and then pelleted. 0.8 ml of polyethylene glycol 1500 (Boehringer Mannheim) was added slowly to the pellet while stirring. After 2 minutes, the cells were diluted slowly with medium, washed, and then plated out in four 24-well plates at 1 ml/well in IMDM + 10% FCS (Gemini Bioproducts Inc.). After 24 and 48 hours, the wells were fed with medium supplemented with HAT (hypoxanthine, thymidine, aminopterin; (Sigma Chemical Co.). Culture supernatants were tested at days 14-21 after fusion for the presence of human IgM RF. Positive wells were then cloned and positive hybridomas selected.

Those skilled in the art will recognize that other myeloma cell lines could be used for fusion with the splenocytes from the transgenic mice. Further, hybridomas 11, 32, 38, 45, 73 and 75 of the invention are

exemplary rather than exhaustively representative of hybridomas which may be produced from the transgenic animals described.

EXAMPLE VI

REACTIVITY OF THE MONOCLONAL ANTIBODIES OF THE INVENTION WITH IDIOTYPIC DETERMINANTS ON IgM PARAPROTEINS AND LACK OF REACTIVITY (TOLERANCE) TO CONSTANT REGION DETERMINANTS

Human IgM RF paraproteins (specifically Glo and VIN) were isolated from patient's with Waldenstrom's macroglobulinemia (a malignancy of IgM secreting lymphoplasmacytoid cells). Isolation was by 45% saturated ammonium sulphate precipitation. These and other IgM paraproteins are also commercially available from sources such as Caltag, Inc., San Francisco, CA; Jackson Immunoresearch Labs, Inc., West Grove, PA; Binding Site Ltd., Birmingham, England; and Tago, Inc., Burlingame, CA.

The AB29 mice were divided into two groups and immunized with either polyclonal human IgM or Glo; Immunization was generally according to the protocol described in Example IV. The mice immunized with Glo received it in an emulsion of Complete Freund's Adjuvant. Each of these mice received a booster injection after 3 weeks of Glo in Incomplete Freund's Adjuvant.

The concentration of any anti-Glo antibodies in sera was measured by enzyme-linked immunoassay (ELISA). The techniques for performing an ELISA are well-known and will not, therefore, be described in detail. Briefly, the assay was performed on plastic microtiter plate wells exposed to a solution of human IgM in phosphate buffered saline (PBS) (20 μg/ml) overnight at 4°C. Any non-specific binding of the IgM was blocked by incubation of

the wells in PBS, 1% bovine serum albumin (BSA) and 0.1% sodium azide for 2 hours at room temperature. The same technique was followed for use in measuring production of anti-VIN and anti-polyclonal IgM antibodies.

Serum samples from all mice were tested at a range of dilutions from 2 x 10^{-2} to 2 x 10^{-7}. Adsorbance was measured at 450 nm. Serum from the mice immunized with the polyclonal IgM was used as the standard in all assays.

Each immunization protocol was also followed in non-transgenic littermates of the transgenic mice as a control.

As shown in Table II below, the mice immunized with Glo produced anti-Glo antibodies (after the booster injection) at 100% of the levels produced by control group (non-transgenic mice). However, the transgenic mice did not produce any discernable response to the polyclonal IgM.

These data indicate that while the AB29 mice were immunocompetent vis-a-vis variable region idiotypic determinants on IgM paraproteins differing from the transgene, they were tolerant to IgM constant region determinants.

The AB8 mice were divided into two groups and immunized with VIN or with human polyclonal IgM. Again, as shown in Table I, the mice produced a strong response (45% of the response produced by nontransgenic mice) to VIN, but had no discernable response to polyclonal IgM. This indicates that the AB8 mice were as tolerant to IgM constant region determinants as the AB29 mice. The AB8 mice also produced anti-idiotypic antibodies, although not to the extent that the AB29 mice produced anti-Glo antibodies. This result could be attributed to a difference in the immunogenicity of Glo and VIN as well

as to a difference in the immunocompetence of AB8 and AB29 mice.

TABLE II

IMMUNE RESPONSES OF TRANSGENIC MICE TO IMMUNIZATION WITH IgM PARAPROTEINS EXPRESSED AS A PERCENTAGE OF THE RESPONSE OF NON-TRANSGENIC LITTERMATES

AB8 mice immunized with VIN				AB29 mice immunized with GLO		
Week	Anti-Vin	Anti-IgM		Week	Anti-Glo	Anti-IgM
0	0	0		0	0	0
2	5	3		2	1	0
4	12	3		4	6	0
6	45	7		24	100	0

Data are expressed as a percentage of the response of normal littermates to the same antigen. Data are calculated from mean results of triplicate mice in each test group.

In order to test the theory that the transgenic response was directed against idiotypic determinants of the immunogen with differed from the endogenous transgene an inhibition ELISA was performed as follows. ELISA plates were coated with Glo protein at 2μg/ml overnight at 4°C. Plates were washed and then blocked for 2 hours at room temperature with phosphate buffered saline/1% BSA and 0.1% azide to prevent non-specific binding. Sera from a transgenic mouse immunized with Glo and a non-transgenic mouse immunized with polyclonal human IgM were pre-titrated for binding to the Glo plates and a concentration of serum chosen which was just below the plateau of the binding curve. Increasing concentrations of either Glo or polyclonal human IgM were then added to

this serum dilution. These mixtures were preincubated overnight at 4°C before being added to a plate coated with Glo. Binding of the sera in the presence of inhibitory protein was then compared to standard curves of the sera in order to determine inhibition of binding.

FIGURE 1 shows that binding of sera from a mouse immunized with Glo could only be inhibited using idiotype identical protein as the inhibitor and not by polyclonal IgM or IgG (indicating that binding was not due to contaminating IgG complexed with RF in the Glo protein preparation). The mice immunized with Glo were also tested for reactivity with other IgM paraproteins. As shown in FIGURE 2, binding to a protein of the same subgroup as Glo (VkIII, Vh3 (HEA)) was only 6% of the level of binding to Glo, thus supporting the conclusion that the response of human IgM transgenic mice immunized with other IgM paraproteins is directed predominantly to idiotypic determinants.

EXAMPLE VII

REACTIVITY OF mAb's PRODUCED FROM AB8 AND AB29 MICE WITH SPECIFIC VARIABLE REGION SUBGROUPS

As noted previously herein, the development of mAb's specific for heavy and light chain idiotypic determinants can facilitate the dissection of the genetic basis for expression of monoclonal rheumatoid factors. This example illustrates how the mAb's described herein can be used to identify variable region subgroup usage by RF's. It also illustrates the production of mAb's specific for idiotypic determinants on these RF's.

Six mAb's (respectively numbered 11, 32, 38, 45, 73 and 75 to identify the hybridoma which produced them) from Glo-immunized AB29 mice or VIN-immunized AB8 mice were separately incubated with the panel of human IgM

paraproteins listed in the left-most column of Table III as follows.

ELISA plates were coated with these paraproteins at 2μg/ml. Culture supernatants from the hybridomas were tested for binding to these coated ELISA plates. In order to make comparisons between the different plates (which may have been coated to different extents by the different paraprotein preparations), a standard curve of a known anti-serum specific for human IgM was applied to each plate and binding expressed relative to this standard.

TABLE III

HUMAN IgM PARAPROTEINS

Protein	Vh group	VL group	MAB 11 Glo	MAB 32 VIN	MAB 38 VIN	MAB 45 Glo	MAB 73 Glo	MAB 75 Glo
Glo	Vh3	VkIIIb	-	-	-	+++	-	+++
Vin	Vh3	VkIIIa	-	++	++	-	-	-
				-	-			
4053	Vh4	VkIIIb	-	-	-	-	-	-
Bat	Vh4	VkIIIb	-	-	-	-	-	-
Lea	Vh4	VkIIIb	+	-	-	-	-	-
A1887	Vh3	VkI	-	-	-	++	-	++
31146	Vh3	VkI	-	-	-	-	-	-
Chr	Vh3	VkI	-	-	-	-	-	-
KD477	Vh3	VkII	-	-	-	-	-	-
Riv	Vh3	VkIII	-	-	-	-	-	-
Scz	Vh3	VkIII	-	-	-	+++	-	++
Joh	Vh3	VkIV	-	-	-	++	-	++
Sim	Vh3	VkIV	+	-	-	-	+	-
293	Vh3	V^	-	-	-	-	-	-
591	Vh3	V^	-	-	-	-	-	-
Mar	Vh1	VkI	+	-	-	-	-	-
725	Vh1	VkIIIb	++	-	-	-	++	-

\+ = 500-2,000 units binding
++ = 2,000-30,000 units binding
+++ = 30,000-300,000 units binding

Two of the mAb's from the AB29 mice reacted strongly (30,000 - 300,000 units binding) with Glo, while two of the mAb's from the AB8 mice reacted moderately (2,000-30,000 units binding) to VIN. Certain mAb's reacted weakly (500-2,000 units binding) to strongly with Vh3 positive paraproteins other than the immunizing paraprotein in each mouse.

mAb's 45 and 75 (from AB29 mice) appear to react with an as yet uncharacterized idiotypic determinant. To explain, the B6 mAb is known in the art to be specifically reactive to the Glo and Scz paraproteins but

not the A1887 paraprotein. On the other hand, the VOH3 mAb is known in the art to be specifically reactive to the A1887 RF paraprotein, but not to Glo or Scz. The 45 and 75 mAb's of the invention bound Glo, Scz and A1887, presumably at an epitope different from those recognized by B6 or VoH3. (For a description of mAb B6, see, Crowley, *et al.*, *Mol. Immunol.*, 27:87, 1990; for a description of mAb VOH3, see, Mageed, *Rheumatol. Int.*, 6:179, 1986).

mAb's 11 and 73 of the invention had similar reactivities. However, these mAb's lost their specificity for Glo (the immunizing antigen) and reacted instead (albeit weakly) with paraproteins from genes restricted in usage of different subgroups. This could be due to undetected damage of the binding site in these mAb's for Glo or to loss of idiotypic specificity due to an escape-like response.

mAb's 32 and 38 produced by fusion of spleens from mice immunized with the VkIIIa, Vh3 paraprotein VIN appear to react only with the immunizing antigen when tested against the panel of IgM paraproteins. This is possibly due to specificity for sequences produced by jointing of VDJ segments during gene rearrangement which are specific for VIN, or alternatively, the panel of paraproteins tested for binding may be too small to detect reactivity with rare epitopes.

EXAMPLE VIII

REACTIVITY OF mAb'S OF THE INVENTION WITH IDIOTYPIC DETERMINANTS ON HUMAN TONSIL CELLS

In order to determine the frequency of cells bearing idiotypes bound by mAb's 45 and 73 in normal secondary lymphoid tissue, human tonsil cell suspensions were stained and analyzed using the fluorescence analysis technique described in Example III. For comparison, these cells were exposed to not only mAb's 45 and 73 of the invention, but also to the B6, VOH3 mAb's and anti-human IgM polyclonal antibodies. The control antibody used was polyclonal mouse IgG.

Table IV shows the percentage of cells staining with each anti-idiotype Mab. From this panel of tonsil samples, approximately 9.0% of the total tonsil cell population and 27% of IgM bearing B cells express immunoglobulin recognized by Mab 45. 6.6% of tonsil cells and 20% of IgM bearing B cells express an immunoglobulin recognized by Mab 75.

TABLE IV

PERCENTAGE OF POSITIVE CELLS IN TONSIL SUSPENSION

Antibody	Tonsil 1	Tonsil 2	Tonsil 3	Mean
B6	5.5 (249)	4.5 (216)	4.6 (185)	4.8 (185)
VOH3	8.0 (366)	5.2 (295)	6.4 (304)	6.5 (322)
LC1	10.4 (193)	9.2 (187)	8.4 (221)	9.3 (200)
Mab45	11.0 (331)	6.9 (292)	9.1 (291)	9.0 (305)
Mab75	8.6 (360)	4.5 (362)	6.6 (325)	6.6 (349)
anti-hu IgM	37.0 (61)	32.2 (74)	30.0 (64)	33.1 (66)

Data are expressed as percentage positive cells and mean fluorescence relative to cells stained with the control antibody.

CLAIMS

1. A method for producing enriched populations of anti-idiotypic antibodies comprising

a. immunizing a non-human animal whose somatic cells will express a transgene which encodes a first immunoglobulin protein which does not detectably react with the animal's endogenous immunoglobulin proteins, wherein the immunization is with at least one immunoglobulin protein that is idiotypically different from the first immunoglobulin protein encoded by the transgene; and,

b. harvesting antibodies produced by cells of the immunized transgenic mammal which are specifically reactive to idiotypic determinants on the immunizing immunoglobulin.

2. A method according to Claim 1 wherein the first and the immunizing immunoglobulins are human immunoglobulins.

3. A method according to Claim 1 wherein the anti-idiotypic antibodies are monoclonal antibodies.

4. A method according to Claim 1 wherein the constant region of the immunizing immunoglobulin is substantially similar to the constant region of the first immunoglobulin.

5. A method according to Claim 1 wherein the first immunoglobulin is an autoantibody.

6. A method according to Claim 5 wherein the autoantibody is a rheumatoid factor.

7. A method according to Claim 1 wherein the immunizing immunoglobulin is an autoantibody.

8. A method according to Claim 7 wherein the autoantibody is a rheumatoid factor.

9. A method according to Claim 1 wherein the immunizing immunoglobulin contains a humanized or chimeric non-human immunoglobulin region.

10. A method according to Claim 9 wherein anti-idiotypic antibodies which are specifically reactive to the humanized or chimeric non-human immunizing immunoglobulin are detected in a biological sample from the immunized transgenic mammal and their quantity is compared to detected levels of anti-idiotypic antibodies which are specifically reactive to a control immunoglobulin which does not contain the humanized or chimeric non-human immunoglobulin region.

11. A method according to Claim 1 wherein the immunized transgenic animal is a rodent.

12. A transgenic non-human animal having a phenotype wherein the animal:

a. expresses a human immunoglobulin protein encoded by a transgene which does not detectably react with the transgenic animal's endogenous immunoglobulin proteins;

b. is immunologically tolerant to constant region determinants on human immunoglobulin proteins;

c. produces antibodies which are specifically reactive with idiotypic determinants on human immunoglobulin proteins in response to the presence of the human immunoglobulin proteins in the transgenic animal.

13. A transgenic animal according to Claim 12 wherein the animal is a rodent.

FIG. 1

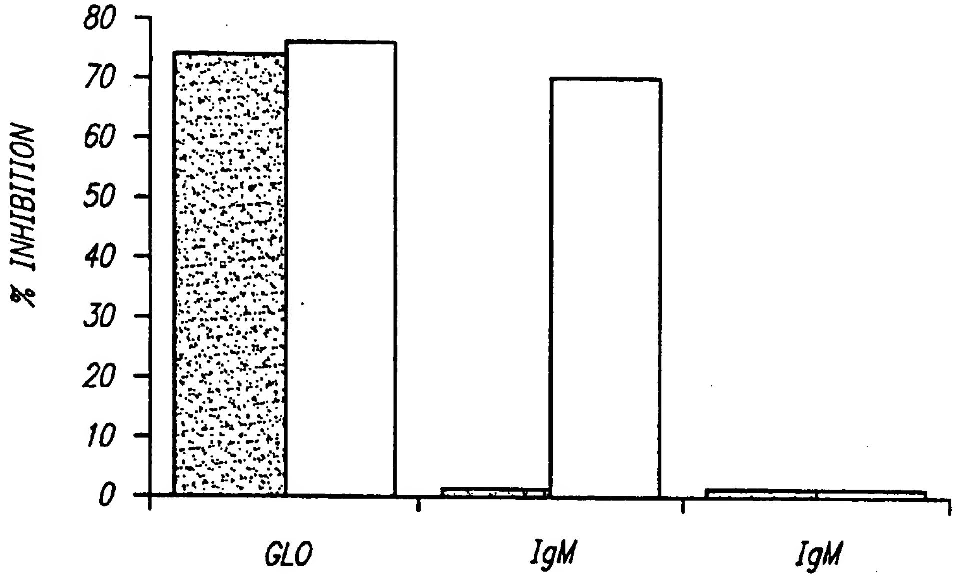
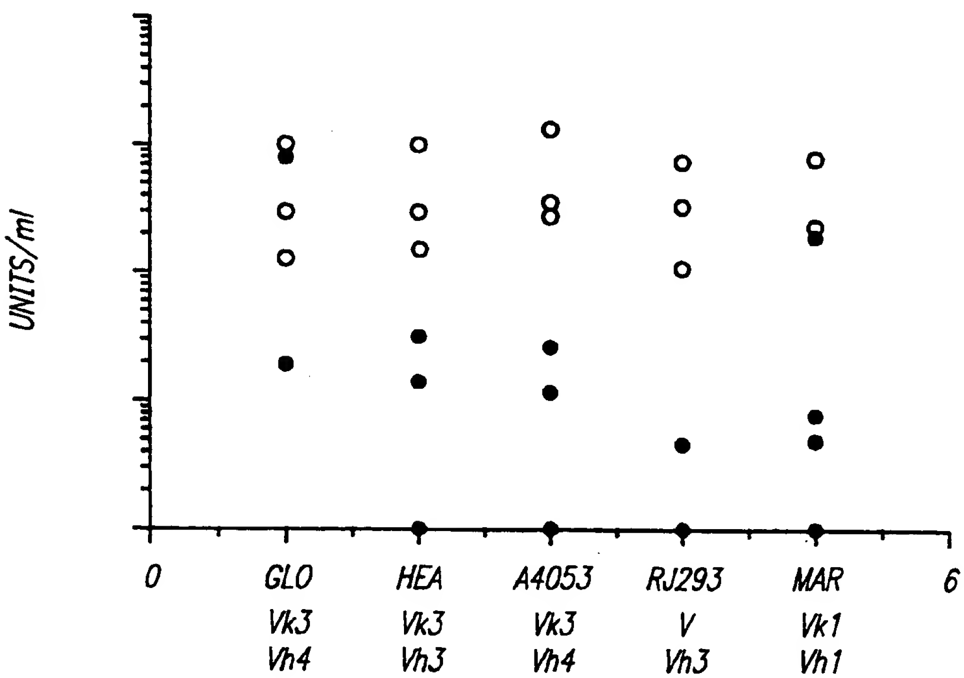

80
70
60
50
40
30
20
10
0
% INHIBITION
GLO
IgM
IgM

FIG. 2


UNITS/ml
0
GLO Vk3 Vh4
HEA Vk3 Vh3
A4053 Vk3 Vh4
RJ293 V Vh3
MAR Vk1 Vh1
6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14467

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :424/9, 85.8; 435/7.1, 7.21, 69.1, 172.3; 530/387.1; 800/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9, 85.8; 435/7.1, 7.21, 69.1, 172.3; 530/387.1; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases searched: APS, EMBASE, MEDLINE, CA, BIOSIS, SCISEARCH
Search terms: antibod?; idiotyp?; transgen?; immun?; monoclon?; constant; autoantibod?; autoimmun?; rf; rheumatoid; factor; mouse; mice; rodent; toleran?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,683,295 (CARSON) 28 JULY 1987, see entire document.	1-13
Y	US, A, 5,034,316 (WEISBART ET AL.) 23 JULY 1991, see entire document.	1-13
Y	JOURNAL OF EXPERIMENTAL MEDICINE, Volume 163, issued June 1986, R.J. Benjamin et al., "Tolerance to rat monoclonal antibodies", pages 1539-1552, see entire document.	1-13
Y	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 1, issued 1991, Tighe et al., "Human rheumatoid factor expression in transgenic mice", page 191, Abstract B217, see entire abstract.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 MARCH 1995	24 MAR 1995

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14467

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/14, 39/395, 49/00; C07K 1/00, 14/00, 14/435, 16/00, 16/18, 16/42; C12P 21/02, 21/08; C12N 15/00; C12Q 1/00